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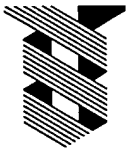

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THE TUMOR NECROSIS FACTOR LIGAND AND RECEPTOR FAMILIES

FLAVIA BAZZONI, PH.D., AND BRUCE BEUTLER, M.D.

TUMOR necrosis factor (TNF) and lymphotoxin-α were isolated more than 10 years ago, on the basis of their ability to kill tumor cells in vitro and to cause hemorrhagic necrosis of transplantable tumors in mice.[1] The complementary DNAs and genes encoding each protein were cloned immediately thereafter.[2,3] Concurrently, a factor known as cachectin was isolated from mouse macrophages, sequenced, and shown to be identical to TNF.[4,5] Cachectin was identified not as a cytolysin, but as a catabolic hormone that suppressed the expression of lipoprotein lipase and other anabolic enzymes in fat.[6-8] Still other studies demonstrated the powerful pro-inflammatory effects of TNF[9,10] and revealed its role as a central endogenous mediator of endotoxic shock.[11,12] Hence, TNF has a broad spectrum of biologic activities.

Because it proved to be highly toxic in animals and humans, TNF did not fulfill initial expectations that it would be useful in the treatment of cancer. However, considerable evidence suggests that overproduction or inappropriate production of TNF may play a part in various chronic inflammatory diseases. Produced largely by macrophages in response to inflammatory stimuli such as lipopolysaccharide, TNF binds to receptors present on virtually all cells throughout the body. TNF, if released systemically in large amounts all at once, modifies the anticoagulant properties of endothelial cells, activates neutrophils, and induces the release of other inflammatory cytokines. These effects culminate in cardiovascular collapse. By contrast, chronic, low-level production of TNF may contribute to the inflammatory response. Bone resorption, fever, anemia, and wasting may all, in some measure, be attributable to TNF[13] (Fig. 1).

What beneficial functions does TNF have? Can its activities be blocked, and if so, at what risk? Above all, how does TNF work? Answers to each of these questions have begun to emerge. They point to regulatory mechanisms that control the biosynthesis of TNF, address the molecular reactions that permit TNF to mediate cell signaling, and suggest a practical means of blocking the activity of TNF for therapeutic effect.

THE TNF-LIGAND AND TNF-RECEPTOR FAMILIES

TNF is one of 10 known members of a family of ligands that activate a corresponding family of structurally related receptors (Table 1). The receptors initiate signals for cell proliferation and apoptosis (programmed cell death). These signals are required for the normal development and function of the immune system. Excessive signaling through some of the receptors can cause severe inflammatory reactions, tissue injury, and shock. Mutations of the genes corresponding to the ligands or the receptors can cause characteristic disturbances of lymphocytes, derangement of the immune response, or autoimmune disease.

All members of the TNF-ligand family are believed to consist of three polypeptide chains. All but lymphotoxin-β (which consists of a single lymphotoxin-α subunit and two lymphotoxin-β subunits) are made up of three identical subunits. All except lymphotoxin-α (which is entirely secreted) and TNF (which is predominantly secreted) are transmembrane proteins that act chiefly through cell-to-cell contact. Nerve growth factor, a dimeric protein, is not actually a member of the TNF-ligand family. Rather, it was apparently adapted in the course of evolution to serve its receptor, a true member of the TNF-receptor family.[33]

All members of the TNF-receptor family are believed to be transmembrane proteins that consist of two identical subunits. The family is defined by a cysteine-rich amino-acid motif that recurs three to six times in the extracellular domain. The cytoplasmic domains vary more than the extracellular domains. Notably, certain receptors contain a 60-residue cytoplasmic sequence known as the "death domain." In the 55-kd TNF receptor and the Fas receptor, this domain is required for the transduction of an apoptotic signal.

With the exceptions of TNF and lymphotoxin-α, each member of the ligand family binds to a specific receptor. TNF and lymphotoxin-α engage two receptors (the 55-kd and 75-kd TNF receptors) with similar affinity. These two cytokines initiate similar (if not identical) biologic responses, although they are produced by different types of cells (lymphotoxin-α is produced exclusively by lymphocytes and natural killer cells, and TNF predominantly by macrophages) in response to different stimuli (antigenic or mitogenic stimuli for lympho-

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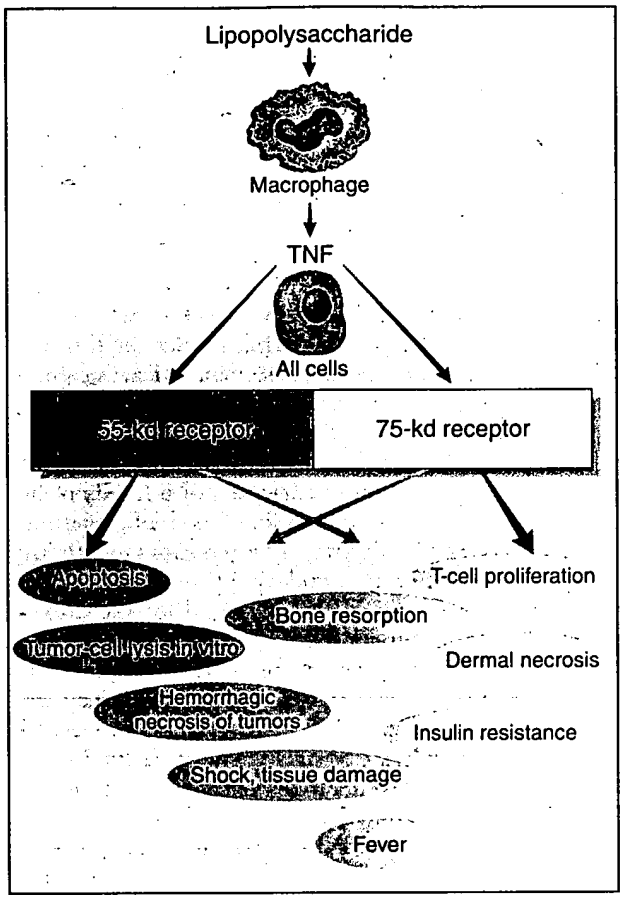

Figure 1. Range of Actions of TNF.
In response to inflammatory stimuli such as lipopolysaccharide, macrophages produce TNF. TNF binds to receptors present on virtually all cells throughout the body, causing a variety of reactions.

toxin-α and lipopolysaccharide or other macrophage-activating agents for TNF).

Functions of TNF Ligands and Receptors

For many years, the role of members of the TNF-ligand and TNF-receptor families in immunity and in the development of the immune system remained speculative. The first indication of their function came from the finding that mutations of the ligands or their receptors can cause disease. Striking examples are the *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) mutations of mice, which were found to specify defects of the Fas receptor (*lpr*) and the Fas ligand (*gld*). These mutant mice have long been taken as models of systemic lupus erythematosus because they have lymphadenopathy and splenomegaly and form autoantibodies.[34,35] In the homozygous state, each mutation causes the accumulation of large numbers of T cells lacking the CD4 and CD8 surface proteins. Heterozygous pairing of the lpr^{CG} allele (CG denotes "complements *gld*") with the *gld* allele also causes lymphoproliferation, suggesting that the products of the two loci might interact with one another.[36]

Further study demonstrated that the lpr^{CG} allele encodes a mutant Fas receptor that can bind its ligand but lacks signal-transducing activity. The classic *lpr* mutation, by contrast, abolishes the expression of the Fas receptor.[37-39] The lpr^{CG} allele contains a point mutation within the death domain of the Fas receptor that prevents signal transduction but does not affect ligand binding.[38] The *gld* allele contains a point mutation that inactivates the Fas ligand.[40] It is widely suspected that mutations of the Fas ligand or its receptor cause a failure of apoptosis in T lymphocytes. This, in turn, leads to massive accumulations of lymphocytes in lymph nodes and spleen and features of autoimmunity.

Humans with mutations of the Fas-receptor gene have also been identified. As in mice with similar mutations, these patients have lymphadenopathy, splenomegaly, and signs of autoimmunity at an early age.[41,42] Mutations of the Fas-receptor gene in humans with lymphoproliferative disease may be associated with the lack of a receptor protein; missense mutations of the gene interfere with signal transduction by the receptor because of faulty interaction with downstream signaling components.

Other members of the TNF-ligand and TNF-receptor families are also important in immune function. For example, the syndrome of X-linked immunodeficiency, in which there are high levels of IgM and low or absent levels of other immunoglobulins, is caused by a mutation in the CD40 ligand.[29,30] Interaction of the CD40 ligand on T cells with the CD40 receptor on B cells mediates immunoglobulin-class switching (the conversion from the production of IgM antibodies to the production of IgG antibodies) and clonal expansion of antigen-responsive B cells. In mice, deletion of the CD40-ligand or CD40-receptor genes results in a phenotype that resembles the disease that occurs in humans.[31,32]

Since naturally occurring mutations that interfere with the function of TNF, lymphotoxin-α, or their two receptors have not been identified, it has been necessary to ablate the genes in mice through a "gene knockout." Both the 55-kd and the 75-kd TNF receptors have been deleted in this manner. Genetically engineered mice lacking the 55-kd TNF receptor are moderately resistant to the lethal effect of lipopolysaccharide but highly susceptible to infection by *Listeria monocytogenes*.[18,19] Mice lacking the 75-kd TNF receptor are moderately resistant to the lethal effect of TNF itself and to dermal necrosis elicited by repeated intradermal injections of TNF.[21] Animals lacking both receptor genes have the sum of these phenotypic effects but no gross developmental effects. It would thus appear that, consistent with the different structure of their cytoplasmic domains, the two TNF receptors fulfill different functions in vivo.

Surprisingly, deletion of both the TNF and lymphotoxin-α genes, which removes the only ligands known to interact with the two TNF receptors, does not yield the same phenotype as the deletion of both receptors. On the contrary, mice in which the lymphotoxin-α gene has been deleted, either alone or in combination with the TNF gene, have no lymph nodes and Peyer's patches and no splenic white pulp. The thymus is grossly preserved.[17]

Table 1. Recently Characterized Members of the TNF-Ligand and TNF-Receptor Families.*

LIGAND	SOURCE OF LIGAND	RECEPTOR	DISTRIBUTION OF RECEPTORS	ABILITY TO INITIATE APOPTOSIS	CYTOPLASMIC MEDIATORS	MUTATION OR KNOCKOUT PHENOTYPE	
						LIGAND	RECEPTOR
TNF and lymphotoxin-α	TNF: macrophages, lymphocytes, keratinocytes, others	55-kd TNF receptor	Many cells	Yes (strong)	TRADD,[14] TRAP-1,[15] 55.11[16]	Both TNF and lymphotoxin-α: absent lymph nodes, decreased lipopolysaccharide responses	Decreased lipopolysaccharide responses; failure to contain listeria or mycobacteria infection[17,18]
	T cells	75-kd TNF receptor	Many cells	Yes	TRAF-1, TRAF-2[19]	Lymphotoxin-α: absent lymph nodes[20]	Decreased lymphocyte proliferation; decreased dermal responses to TNF; decreased TNF-induced lethality[21]
Lymphotoxin-β heteromer	T cells, others	Lymphotoxin-β receptor (TNF-receptor–related protein)	T cells, B cells, others	Yes	LAP-1 (CRAF-1)[22]	ND	ND
Fas ligand	T cells	Fas receptor	Many cells	Yes (strong)	Tyrosine phosphatase (FAP-1),[23] FADD (MORT-1)[24]	Lymphoproliferation	Lymphoproliferation
Nerve growth factor	NA	Nerve growth factor receptor	Neurons, others	No	ND	NA	Neuropathy[25]
CD40 ligand	T cells	Nerve growth factor receptor	B cells, T cells	No	CRAF-1, CAP-1[26-28]	X-linked immunodeficiency with increased IgM and decreased or absent IgG, IgA, IgD[29-31]	X-linked immunodeficiency with increased IgM and decreased or absent IgG, IgA, IgD[32]
CD27 ligand	T cells	CD27	T cells	ND	ND	ND	ND
CD30 ligand	T cells	CD30	T cells	ND	ND	ND	ND
OX-40 ligand	T cells	OX40	T cells	ND	ND	ND	ND
4-1BB ligand	T cells	4-1BB	T cells	ND	ND	ND	ND

*Other members of these two families include the CD27, CD30, OX-40, and 4-1BB ligands and receptors. TRADD denotes TNF-receptor–associated death domain, TRAP-1 TNF-receptor–associated protein 1, TRAF TNF-receptor–associated factor, LAP-1 latent membrane protein type 1–associated protein, CRAF-1 CD40-receptor–associated factor 1, ND not determined, FAP-1 Fas-associated protein 1, FADD (or MORT-1) Fas-associated death domain, NA not applicable, and CAP-1 CD40-associated protein 1.

These results may reflect the fact that the lymphotoxin-α gene is required for the formation of not only the lymphotoxin-α homotrimer but also the lymphotoxin-β heteromer.[43] Interaction between the lymphotoxin-β heteromer and the lymphotoxin-β receptor is probably required for lymph-node development.[17,44,45] Lymph nodes fail to develop in lymphotoxin-α–deficient mice despite the fact that the lymphoctye subtypes are distributed normally in peripheral blood. Lymphotoxin-α–deficient lymphocytes are readily incorporated into the lymph nodes of normal mice, and lymphotoxin-α–positive lymphocytes fail to induce the development of lymph nodes in lymphotoxin-α–deficient mice. Therefore, the failure of lymphotoxin-β heteromer to be expressed on the surface of a nonlymphoid cell, such as a lymph-node stromal element, would seem to preclude normal development of peripheral lymphoid tissues.[46]

How the Receptors Work

Interactions between TNF and its receptors are presumably typical of interactions between the other ligand–receptor pairs in these two molecular families. Trimeric ligands of the TNF family may cause aggregation or clustering of receptor subunits, thereby triggering a cellular response, since antibodies against either of the two TNF receptors mimic the actions of TNF.[47,48] Support for the aggregation model (Fig. 2A) is bolstered by the crystal structure of lymphotoxin-α, which forms a complex with extracellular-domain fragments of the 55-kd TNF receptor,[49] in which three receptor fragments crystallize with each lymphotoxin-α trimer.

Crystallization of the extracellular domain of the 55-kd receptor in the absence of ligand yields a dimeric protein, in which each subunit is arranged head to head with the other. The 55-kd receptor on the cell surface might thus be dimeric in the absence of TNF. A dimeric conformation would allow for a hexagonal array of dimeric receptors and trimeric ligand molecules, which could generate signals by making contacts between their cytoplasmic domains (Fig. 2B). Alternatively, each dimeric receptor might act as an independent "molecular switch," undergoing rearrangement after engaging the trimeric ligand[50] (Fig. 2C).

The molecular-switch model is strongly supported by the effect produced by substituting an erythropoietin-receptor extracellular domain for the extracellular domain of either TNF receptor. This leads to constitutive signaling activity in the cell (i.e., a perpetual "on" state).[50] The conformation of the receptor dimer, rather than dimerization itself, is thus the critical issue in signal transduction. Moreover, an essential attribute of the extracellular domain of the TNF receptor is its ability to prevent signal transduction in the absence of a ligand. If the extracellular domains are removed,[51] grossly modified,[50] or displaced by an antibody,[47,48] signaling is initiated.

One of the most intriguing actions of TNF is the induction of apoptosis. Apoptosis is almost certainly relevant to some of the toxic effects of TNF, such as shock

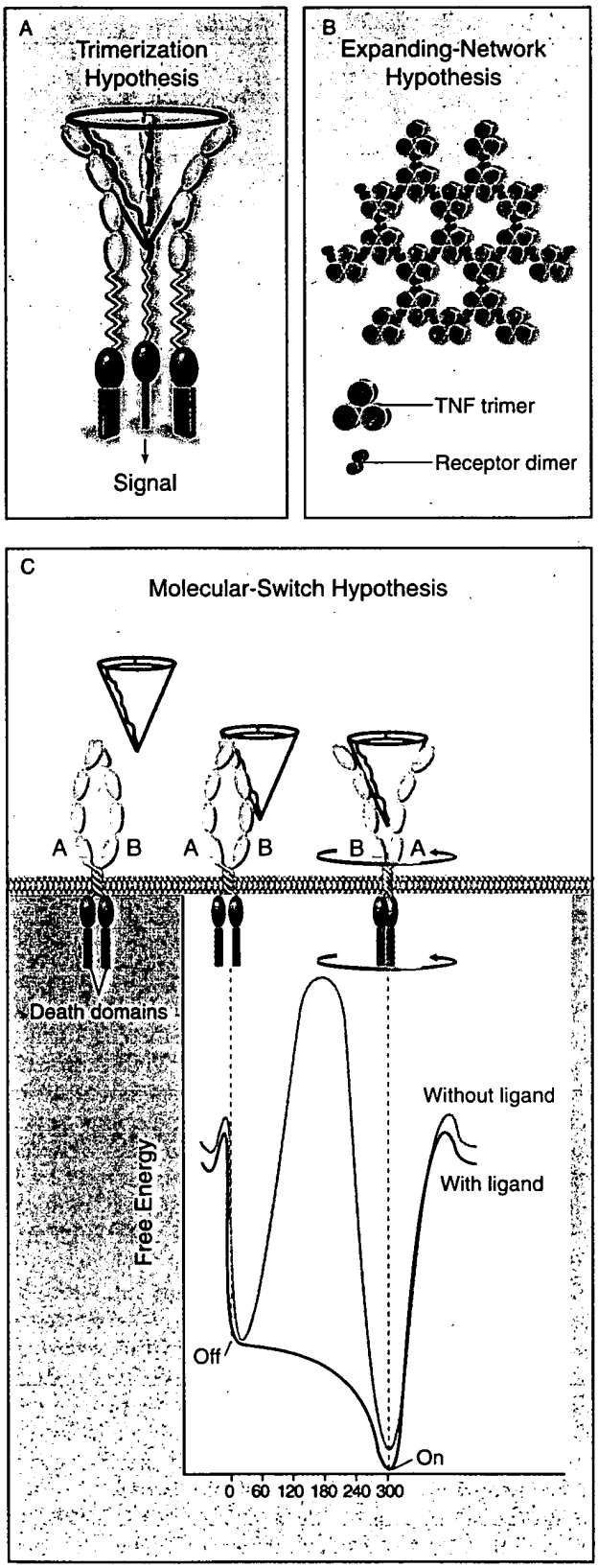

Figure 2. Three Models of the Molecular Events in TNF Signaling.

Panel A shows the trimerization hypothesis. In this model, the juxtaposition of three receptors results from their binding of a single TNF trimer. The resultant complex generates an activating signal.

Panel B shows the expanding-network hypothesis. A growing hexagonal array of TNF trimers bound to TNF-receptor dimers takes account of the dimeric structure of TNF receptors free of ligands and the capacity of each ligand molecule to engage three receptor subunits. "Capping" of receptors would trigger a biologic response.

Panel C shows the molecular-switch hypothesis, the most favored model. In this model each receptor dimer is an activatable unit. Receptor activation occurs in response to two events. First, the ligand binds to subunit B of the receptor. Second, subunit A disengages from subunit B, which permits binding of the receptor to a second available site on the ligand surface. These events cause conformational changes within the cytoplasmic domain of the receptor, leading to signal transduction. Specifically, the death domains of the 55-kd TNF receptor or Fas receptor might undergo homodimerization. The graph shows an imaginary profile of the free energy associated with conformational changes between the "off" and "on" states of the receptor. It is supposed that a large activation-energy barrier prevents transition from the off to the on state in the absence of ligand and that TNF effectively catalyzes this transition, thereby "throwing the switch." Moreover, as depicted here, the free energy of the on state, which presumes a stable association between TNF and the receptor, may be substantially lower than the free energy of the off state, and this may be irreversible.

and inflammation. TNF-induced apoptosis may also have physiologic relevance, as does the apoptosis induced by the Fas receptor. It is possible, for example, that TNF-mediated apoptosis of infected cells helps protect the host. It is likely that both TNF receptors participate in cell death, although the 55-kd receptor is more potent than the 75-kd receptor. Since the cytoplasmic domains of the two receptors are structurally different, each must initiate apoptosis through distinct mechanisms.

Proteins Used by the TNF-Receptor Family for Signal Transduction

Several proteins that bind intracellularly to receptors of the TNF family have been identified (Fig. 3). The first to be identified were the TNF-receptor–associated factors (TRAFs), which have high affinity for the 75-kd TNF receptor.[20] Their biologic function is unknown. Most contain two protein motifs termed the "zinc finger" and "ring finger." TRAF-2 may form a homodimer with itself or a heterodimer with TRAF-1. TRAF-2, TRAF-3 (also known as CD40-receptor–associated factor 1 [CRAF-1] or latent membrane protein type 1–associated protein [LAP-1]), and the closely similar CD40-associated protein 1 (CAP-1) bind not only to the 75-kd TNF receptor, but also to the lymphotoxin-β receptor and the CD40 receptor. Moreover, TRAF-3 also binds to latent membrane protein type 1, a protein of the Epstein–Barr virus that is essential to cell transformation.[22]

An entirely different class of cytoplasmic proteins bind to the 55-kd TNF receptor and the Fas receptor. These proteins are important in transducing signals for programmed cell death. The Fas-associated death domain (FADD),[52] also called MORT-1,[24] the TNF-receptor–associated death domain (TRADD),[14] and the receptor interacting protein (RIP)[53] bind to the Fas receptor, the 55-kd TNF receptor, and both receptors, respectively. Each of these proteins contains a version of the death domain found within the receptors them-

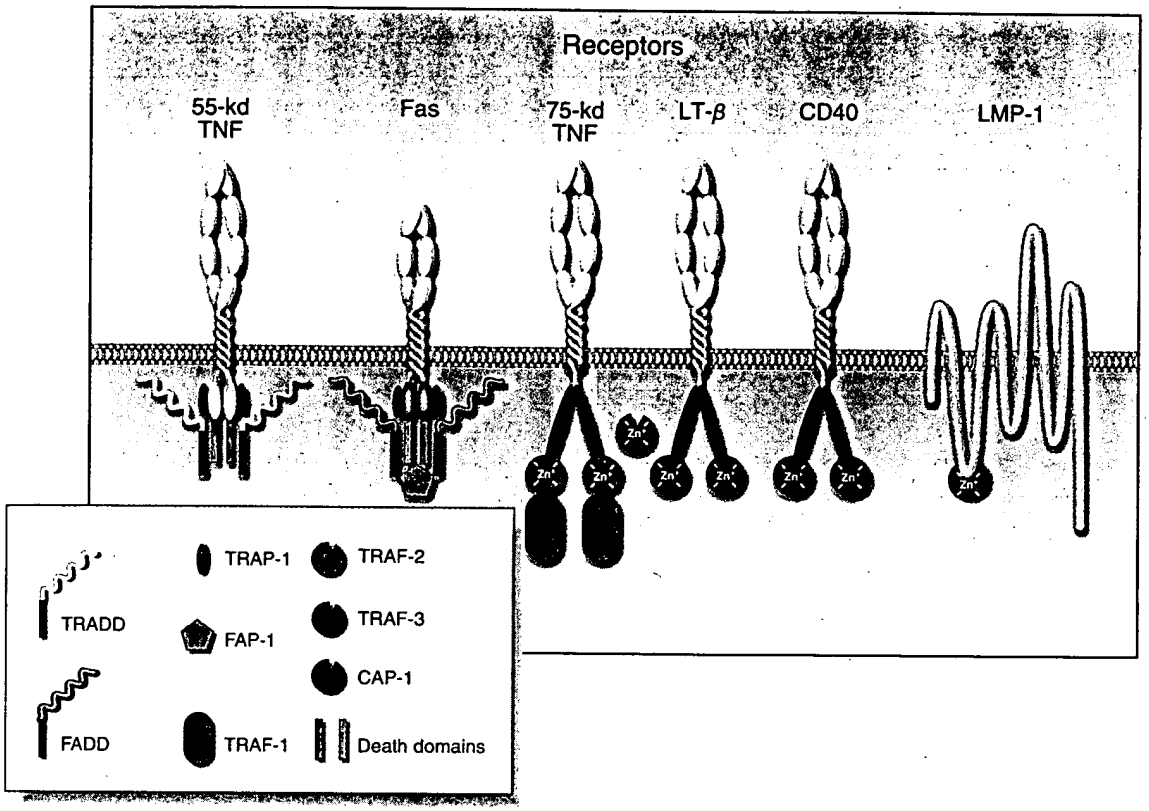

Figure 3. Proteins That Bind the Cytoplasmic Domains of Receptors for TNF, Lymphotoxin-α, Lymphotoxin-β (LT-β), CD40 Ligand, Fas Ligand, and Latent Membrane Protein Type 1 (LMP-1).

Lymphotoxin-α and TNF bind to the same 55-kd and 75-kd TNF receptors. Proteins and domains with similar structures or functions, such as TNF-receptor–associated death domain (TRADD), receptor-interacting protein (RIP), Fas-associated death domain (FADD, or MORT-1) molecules, and the death domains of the Fas and 55-kd TNF receptors, are generally portrayed by identical shapes but in different colors. Zn is used to denote proteins known to contain ring-finger and zinc-finger motifs that bind to the 75-kd TNF receptor, lymphotoxin-β receptor, and CD40-ligand receptor. TNF-receptor–associated factor type 1 (TRAF-1) lacks a zinc-finger motif but binds to TRAF-2, which has both ring-finger and zinc-finger motifs. CD40-associated protein 1 (CAP-1), which is structurally very similar to TRAF-3, is thought to be capable of replacing TRAF-3 as a binding partner for the lymphotoxin-β receptor and CD40-ligand receptor. LMP-1 is a plasma-membrane protein that spans multiple domains and is encoded by the genome of the Epstein–Barr virus. It has no homology to members of the TNF family. Not all possible combinations and interactions are shown. In addition, certain binding proteins that were not discussed in the text belong neither to the zinc- and ring-finger family nor to the death-domain family of transducers. These include TNF-receptor–associated protein 1 (TRAP-1), a heat–shock protein analogue[15]; the 55.11 protein, a proteasome regulatory subunit[16]; and Fas-associated protein 1 (FAP-1), a protein tyrosine phosphatase that binds to the Fas receptor near its carboxy terminal and is thought to decrease the intensity of signals generated by this receptor.[23]

selves. This motif permits interaction between receptor and transducer molecules.

FADD[24,52] is a proximal transducer of the apoptotic activity of the Fas receptor, with which it forms a heterodimer. Engagement of the ligand causes the release of homodimeric FADD, which relays the death signal to the cytoplasm. FADD is incapable of forming heterodimers with receptors encoded by the mutant *lpr*^{CG} gene. Hence, it is at precisely this level that the *lpr*^{CG} mutation interrupts signaling.

Acting in an analogous fashion, TRADD is a proximal transducer of apoptosis mediated through the 55-kd TNF receptor.[14] RIP appears to serve both the 55-kd TNF receptor and the Fas receptor. The transducers may interact with distinct targets downstream from the receptor. Although overexpression of any of the transducers can initiate cell death, different portions of each protein act to carry the apoptotic signal forward within the cytoplasm.[54] Moreover, RIP is far larger than either TRADD or FADD and contains a kinase domain.[54]

In accordance with the molecular-switch hypothesis, a receptor modified by the binding of ligand might effectively catalyze the formation of homodimeric FADD, TRADD, or RIP (Fig. 4), causing conformational changes in these molecules that lead to further reactions in the signaling cascade. This scenario is consistent with the observation that modified receptors are constitutively activated in the absence of ligand.[50] A detailed discussion of the distal components of the cascade by which the death signal is conveyed is beyond the scope of this review.[55-58]

Clinical Effects of TNF and Lymphotoxin-α Blockade

Extensive clinical trials have been performed to test monoclonal antibodies that selectively neutralize TNF (but not lymphotoxin-α) in the treatment of septic shock. To date, double-blind, controlled studies have not documented a substantial benefit.[59,60] However, septic shock is a fulminant disease in which considerable

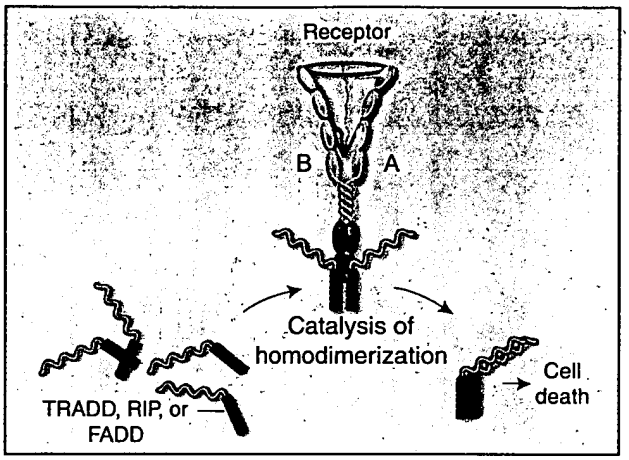

Figure 4. Proposed Catalysis of the Homodimerization of TRADD, RIP, and FADD by the Cytoplasmic Domain of Ligand-Activated Receptor.

According to the molecular-switch model presented in Figure 2C, ligand binding causes a conformational change in the cytoplasmic domain of a dimeric receptor. The relation between the death domains (red rectangles) then favors the formation of TRADD, RIP, or FADD (or MORT-1) homodimers, permitting signal transduction to be carried downstream to components of the pathway that results in cell death. Homodimers do not form spontaneously unless TRADD, RIP, or FADD is artificially expressed at very high levels in the cell. A and B refer to the subunits of the receptor.

damage may already have occurred before the initiation of therapy. The different goal of treating chronic inflammatory diseases, in which slow, continuous tissue damage may occur owing to the presence of TNF, has met with greater success. For example, anti-TNF antibodies lessen pain, joint swelling, anemia, and elevated erythrocyte sedimentation rates in patients with rheumatoid arthritis.[61-63] Encouraging results have also emerged from the use of anti-TNF antibodies in the treatment of Crohn's disease.[64]

Therapy with anti-TNF antibodies has several shortcomings, however. First, anti-TNF antibodies do not prevent signaling of either of the homotrimeric TNF receptors, since they do not neutralize lymphotoxin-α. Second, the antigenicity of murine monoclonal antibodies, and even humanized monoclonal antibodies, may preclude long-term therapy. Third, complement fixation and other reactions related to the formation of immune complexes might be harmful in patients receiving anti-TNF antibodies. Finally, the concentration of monoclonal anti-TNF antibody required to achieve neutralization is very high; consequently, blockade might be expensive to maintain.

The use of chimeric inhibitor molecules (Fig. 5), in which the extracellular domain of the TNF receptor is spliced to an immunoglobulin heavy-chain fragment, might circumvent all these problems. Such molecules are as stable in vivo as immunoglobulins. Because they are composed of two nonantigenic elements, they are minimally antigenic. Their mode of action is highly specific, since their binding domain is a receptor. Moreover, they are broad-spectrum agents, since they neutralize both TNF and lymphotoxin-α, preventing the activation of both TNF receptors. On a weight basis, chimeric inhibitors are far more potent than monoclonal anti-TNF antibodies, because the receptor has a far higher affinity for the ligand than does the antibody.[65,66]

Chimeric TNF inhibitors have been produced in mice with adenoviral vectors. Milligram quantities of the protein were present per milliliter of plasma,[67] causing complete neutralization of TNF and lymphotoxin-α in vivo. One day, gene transfer might be used to produce the inhibitor protein in patients with inflammatory diseases related to the overproduction of TNF.

The Future of TNF

The breadth of actions ascribed to TNF is remarkable (Fig. 1). The molecule is one of the best-characterized gateways to apoptosis. It is essential for defense against intracellular pathogens. It is a pro-inflammatory mediator that can, when overproduced, cause shock and tissue injury. Low levels of TNF may account for the state of insulin resistance that contributes to the development of type II diabetes mellitus.[68-75] Given the complexity of the biomedical problems that involve TNF, it is fortunate that the groundwork for a comprehensive understanding of this cytokine has been laid with the identification of its receptors and many of their signaling intermediates.

The lupus-like state that follows abrogation of the function of the Fas ligand or receptor suggests that some autoimmune disorders could involve defects in the Fas- or TNF-signaling axes. The observation that the administration of TNF attenuates or prevents some autoimmune diseases in animals supports this view.[76-79] TNF may never prove useful in the treatment of widely disseminated cancer, but the insight into tumor-cell vulnerability gained through studies of TNF signal transduction may ultimately yield novel chemotherapeutic approaches.

Ways to block the biosynthesis or action of TNF could have important clinical applications. TNF has served as the principal end point in most studies of endotoxin signal transduction. It is likely that drugs impairing each step of that process will soon be tested for antiinflammatory efficacy. TNF and lymphotoxin-α can already be neutralized, and neutralization of other members of the ligand family is being explored.[80] Thus, new and highly specific approaches to the treatment of inflammatory disease may soon be at hand.

Discussion

DR. JEFFREY FLIER: What is the relative role of transcription and translation in vivo in activating TNF? Can you discuss your work on transgenic mice that relates to this issue?

DR. BEUTLER: The biosynthesis of TNF is controlled by two switches — one transcriptional and one translational — that work in concert with each other. The activation of macrophages by lipopolysaccharide causes a 50-fold increase in TNF messenger RNA and a 100-fold increase in translational efficiency. The rate of production of TNF protein increases by a factor of several thousand. My colleagues and I created transgenic mice bearing a reporter construct in which the TNF coding sequence was replaced by DNA coding

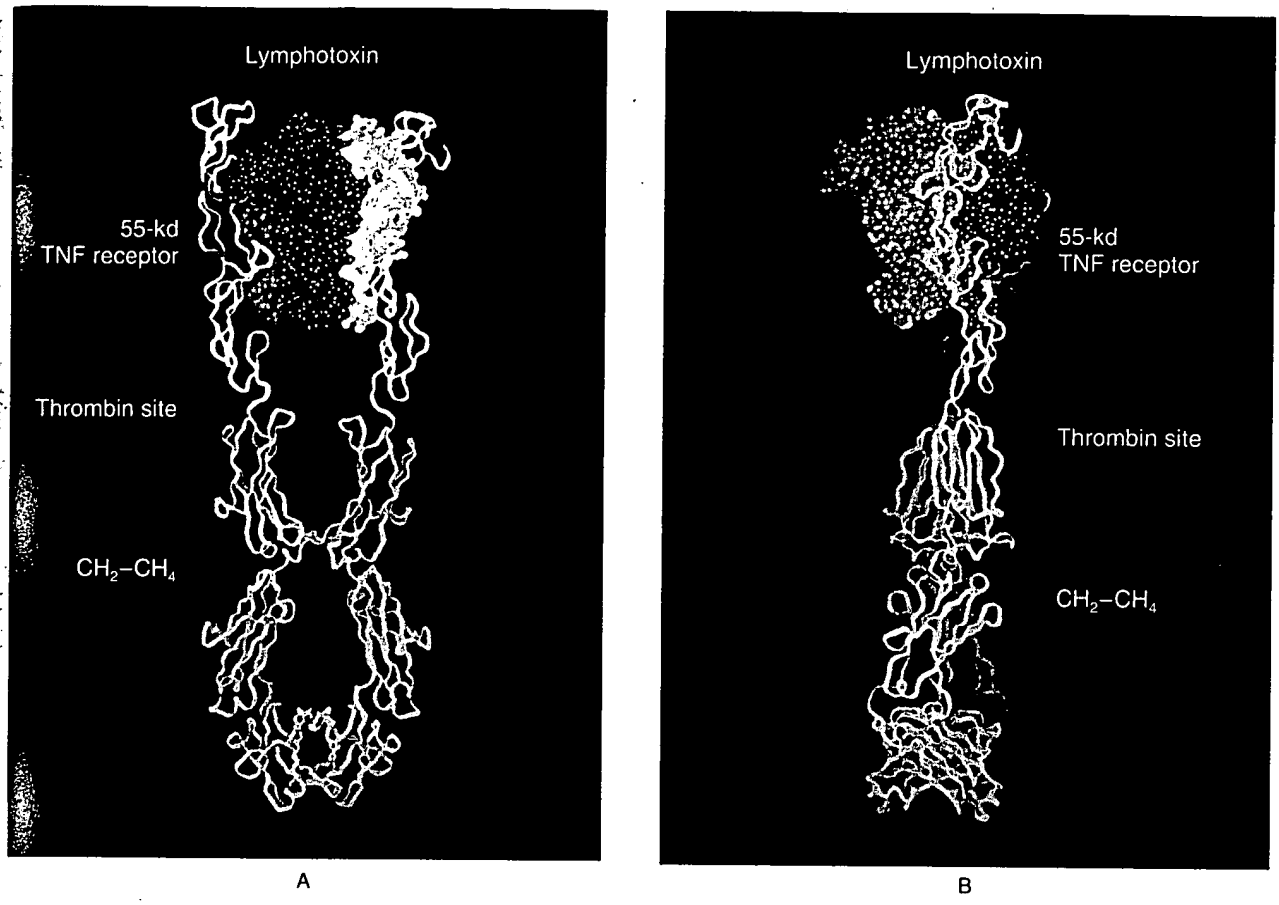

Figure 5. Predicted Tertiary and Quaternary Structures of TNF-Inhibitor Molecules Created by Fusing the Extracellular Domain of the TNF Receptor to Domains CH_2, CH_3, and CH_4 of an IgG Heavy Chain.

Two views of the molecule, binding to a lymphotoxin-α trimer, are shown (Panels A and B). The binding of lymphotoxin-α to the extracellular domain of the 55-kd TNF receptor was reported by Banner et al.[49] and is used to model this portion of the chimeric protein (yellow). The IgG moiety is blue. The lymphotoxin-α trimer subunits are green, white, and red. This model was assembled by Dr. Stephen Sprang.

for the marker enzyme chloramphenicol acetyltransferase, or CAT. The CAT-reporter transgene behaved rather like the TNF gene itself and revealed to us that TNF is produced constitutively in the thymus and trophoblast. However, its function in these tissues remains unknown.

A PHYSICIAN: What signals do the ring- and zinc-finger proteins convey?

DR. BEUTLER: There is a tendency to think that they may transduce mainly proliferative signals. The receptors to which they bind have largely proliferative functions. This is true of the 75-kd TNF receptor and the CD40-ligand receptor. It may also be true of the lymphotoxin-β receptor. Common transducers may therefore produce a common effect, in what amounts to cross-communication among the three. On the other hand, each receptor does have unique properties, and it is unlikely that any one of them can truly substitute for another.

A PHYSICIAN: What do we know about how TNF actually causes apoptosis? Specifically, what makes tumor cells so sensitive to it?

DR. BEUTLER: It is still not clear why the cells are so sensitive. Almost any kind of cell can be lysed by TNF in the presence of protein-synthesis inhibitors. The general thinking has been that tumor cells lack a short-lived protective factor that enables normal cells to escape the cytolytic effect of TNF. Members of the family of proteases containing interleukin-1β–converting enzyme have been implicated as downstream transducers in the TNF and Fas signaling pathways. Inhibitory members of the Bcl-2 family of proteins, such as Bak, may also be involved. However, a clear and complete biochemical pathway has yet to be established. Therefore, we cannot say precisely why certain tumor cells are sensitive to TNF, nor do we know what advantage this might confer.

DR. VIKAS SUKHATME: Is it possible to make TNF antagonists?

DR. BEUTLER: True antagonists have not yet been designed, although theoretically it should be possible to achieve this. A heteromeric form of TNF, with only one site capable of binding to the receptor, might fulfill such a function.

DR. FLIER: Could you tell us whether the pharmaceutical industry is using the TNF inhibitors that you developed?

DR. BEUTLER: The chimeric inhibitors have now been produced for clinical use by a number of companies. They are being tested for efficacy in the treatment

of rheumatoid arthritis. Already, proof of principle has been supplied by the striking remissions induced by anti-TNF antibodies. There is reason to believe that chimeric inhibitors will perform even better, given their activity against lymphotoxin-α as well as TNF, their extraordinary affinity for these ligands, and their relative lack of antigenicity.

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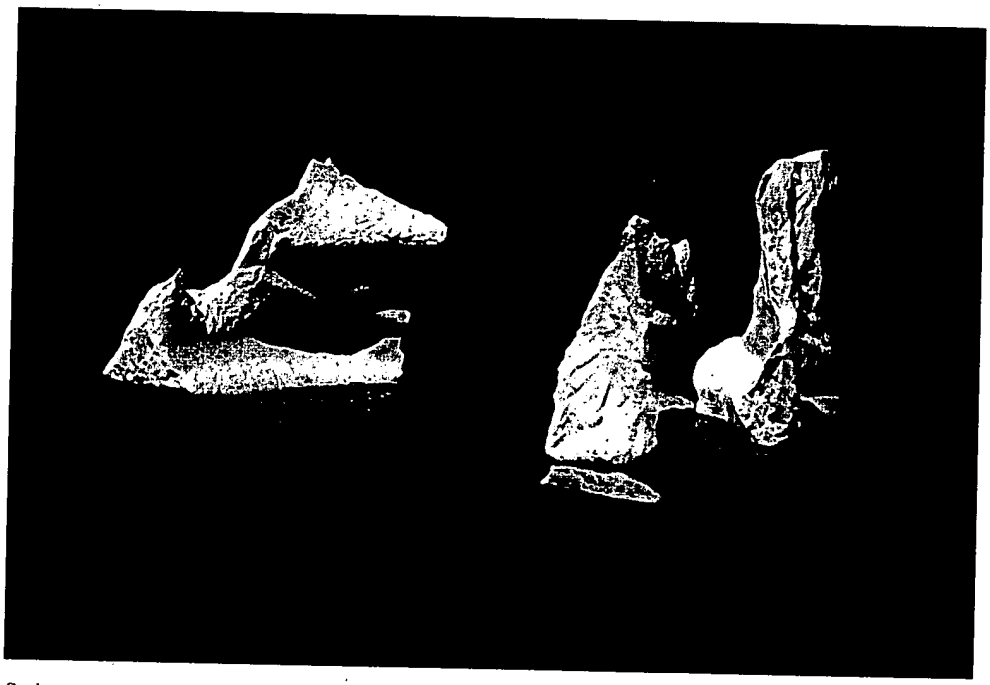

Sculptures in Ice

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